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Multifocal structured illumination optoacoustic microscopy

Zhenyue Chen[a], Xosé Luis Deán-Ben[c], Ali Özbek[a,b], Johannes Rebling[c], and Daniel Razansky[a,b,c,d,*]

[a] Institute for Biological and Medical Imaging, Helmholtz Center Munich, Neuherberg, Germany
[b] Faculty of Medicine, Technical University of Munich, Germany
[c] Faculty of Medicine and Institute of Pharmacology and Toxicology, University of Zurich, Switzerland
[d] Institute for Biomedical Engineering and Department of Information Technology and Electrical Engineering, ETH Zurich, Switzerland

ABSTRACT

Optoacoustic imaging is a highly scalable and versatile method that can be used for optical resolution (OR) microscopy applications at superficial depth yet can be adapted for tomographic imaging with acoustic resolution at centimeter penetration scales. However, imaging speed of the commonly employed scanning-based microscopy methods is slow as far as concerned with acquisition of volumetric data. Herein, we propose a new approach using multifocal structured illumination in conjunction with a spherical matrix ultrasonic array detection to achieve fast volumetric optoacoustic imaging in both optical and acoustic resolution modes. In our approach, the laser beam is raster scanned by an acousto-optic deflector running at hundred hertz scanning rate with the beam then split into hundreds of mini-beams by a beamsplitting grating, which are subsequently focused by a condensing lens to generate multifocal structured illumination. Phantom experimental results show that 10 x 10 x 5 mm^3 volumetric imaging can be accomplished with spatial resolution around 29 μm. We believe by further speeding up the data acquisition in the further, the system will be operated in full power, making it possible to study functional, kinetic and metabolic processes across multiple penetration scales.

Keywords: optoacoustic microscopy, optoacoustic tomography, multifocal structured illumination, acousto-optic deflector, beamsplitting grating

1. INTRODUCTION

Optoacoustic imaging is a highly scalable and versatile method that can achieve optical resolution microscopy at superficial depths through the use of a focused illumination; yet can be adapted for tomographic imaging with ultrasonic resolution at much greater depths of many millimeters to centimeters where the excitation light is fully diffuse [1, 2].The most recent optoacoustic tomography (OAT) technology employs spherical matrix arrays, parallel acquisition hardware, GPU-based data processing, and fast laser tuning systems to enable real-time acquisition and visualization of volumetric multi-spectral optoacoustic data [3-5]. This five-dimensional (5D) optoacoustic tomography technology that can render spectrally-enriched information from entire scattering tissue volumes with spatial resolution of 70-200μm, field of view (FOV) of up to several cm^3 and an effective frame rate of 100 volumes per second, limited by the pulse repetition frequency of the laser employed. As compared to OAT, optical resolution optoacoustic microscopy (OR-OAM) has the advantageous combination of diffraction limited optical resolution on the order of several μm. However, high-resolution OR-OAM systems are using point-by-point scanning to render 3D images [6-8], resulting in a slow imaging speed as far as concerned with acquisition of volumetric data, which greatly restricts applications in visualization of dynamic biological processes.

To improve the imaging speed of OR-OAM, a number of solutions have been previously proposed. Song et al. [9] reported a multifocal OR-OAM method. A line of 20 microlenses from a microlens array were employed to generate

* Corresponding author: daniel.razansky@uzh.ch

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the multifocal optical illumination in conjunction with a linear ultrasonic array detection. The imaging speed was increased by 20-fold. However, due to the difficulties in confocal alignment, the sample was scanned, making the system inconvenient for practical applications. Hu et al. [10] proposed a second-generation OR-OAM system by translating the imaging head instead of the living object which accelerated the scanning speed by a factor of 5. Yao et al. [11] presented a wide field fast-scanning OR-OAM system by using a lab-made water-immersible microelectromechanical systems scanning mirror. To maintain confocal alignment and detection sensitivity, both the excitation laser beam and the resultant acoustic beam had to be scanned. Xia et al. [12] proposed a two dimensional multifocal OR-OAM method by introducing a microlens array and a 2D imaging system. However, the data of the proposed system couldn't be acquired within one laser pulse and the large number of optical foci required a high power laser which in return limited the pulse repetition rate, making the system slower than conventional OAM systems. Liang et al. [13] developed a random-access OR-OAM using a digital micro-mirror device (DMD). However, to increase the frame rate, only a small region of interest could be imaged. Due to low light utilization efficiency of DMD, a high power laser source was required in this method. An all-optical tomographic optoacoustic imaging system based on a Fabry-Perot interferometric ultrasound sensor has been reported which can achieve tomographic information along a planar surface using unfocused point detection, resulting in accurate 3D reconstructions yet at the expense of relative long scanning time [14, 15].

Herein, we propose a new approach using **m**ultifocal **s**tructured **i**llumination in conjunction with a spherical ultrasonic array detection to achieve a highly scalable high-speed optoacoustic imaging scheme, i.e., MSIOAM. It will be able to perform both OR-OAM and OAT, i.e. operate at multiple penetration scales by gradually exchanging microscopic optical resolution in superficial tissues with acoustic resolution at diffuse (macroscopic) depths.

2. METHODS

2.1 System setup

Fig. 1 depicts the schematic diagram of the proposed multifocal structured illumination optoacoustic microscopy (MSIOAM) system. A Q-switched, diode-pumped Nd:YAG laser (model: IS8II-E, EdgeWave, Germany) operating at 532 nm wavelength was used for optoacoustic signal excitation. The pulse repetition frequency (PRF) of the laser is adjustable and up to 10 kHz. The laser beam was first scanned by a two dimensional acousto-optic deflector (2D AOD) (AA Opto-Electronic, France) and then guided into a customized beamsplitting grating (Holoeye GmbH, Germany) to generate multiple mini-beams. A series of gratings were manufactured with different diffraction orders, such as -7 ~ +7 orders and -10 ~ +10 orders to meet the requirements for different studies. The diffraction efficiency of these gratings is ~74% and intensity uniformity within 10% for all the diffraction orders. The angle between adjacent diffraction orders is 0.57°, smaller than the maximum scanning range of 2.292° the AOD. These hundreds of split mini-beams were firstly relayed by Lens 1 and then focused by Lens2 onto the sample to generate multiple foci [16]. The excited acoustic signal was collected by a custom-made ultrasonic array detector (Imasonic SaS, France) which consists of 512 piezocomposite elements with ~2.5 mm diameter, 5 MHz central frequency and ~100% detection bandwidth at full width at half maximum (FWHM). The elements are uniformly distributed on a spherical surface with 40 mm radius and 140° angular coverage (1.3π solid angle) [17]. The data was sampled by a 512-channel data acquisition system (DAQ) at 40 mega-samples per second and then transferred to the computer.

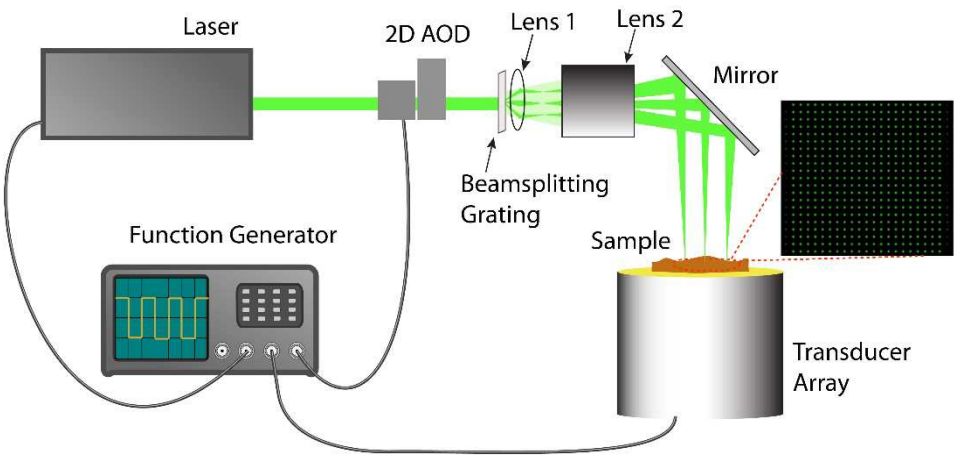

Fig. 1 Schematic drawing of the system setup. During data acquisition, the laser pulse, AOD beam deflection and acoustic signal detection are synchronized with external trigger signals.

2.2 Phantom experiment

After setting up the system as shown in Fig. 1, carbon fibers with 6.9 μm diameter (SIGRAFIL C T24-5.0, Germany) were employed as the sample. They were embedded into 1.3% agar phantom at different depths. The ultrasonic array detector was also filled with 1.3% cooked agar liquid to couple the acoustic signal. After the coupling agar in the array detector got solid, the carbon fiber phantom was put on top of it and was kept close to the focal plane of the array detector. In the experiment, a 15 x 15 beamsplitting grating, a 35 mm plano-convex lens (Lens 1) and a Nikkor 35mm f/2D lens (Lens 2) were employed to generate the multifocal structured illumination pattern. The effective pulse energy after the beamsplitting grating was measured to ~500 μJ. During the experiment, laser pulsing, AOD beam scanning and data acquisition were synchronized with a function generator. Due to the limited speed of data acquisition and transfer, in our preliminary experiment the frame rate was set to 100 Hz. To acquire one complete image of the imaged region, the illumination pattern was scanned 100 x 100 steps, meaning that it took 100 s to finish data acquisition.

2.3 Image reconstruction

After data acquisition, image reconstruction was performed with MATLAB. Firstly, filtered back projection (BP) method [18] was implemented to each scanning frame to obtain the 3D dataset. Secondly, regional maxima were localized within the entire reconstructed 3D dataset from the last step. Since the information of the illumination pattern, including spot size and locations was roughly known, the real acoustic signals could be recognized and localized more easily and robustly. Thirdly, virtual acoustic pinholes were applied to the localized signals. The voxels from each spot were zeroed out in the peripheral region with only the centroid voxel kept and set as the signal. Finally, all the extracted signals from last step were superimposed to form the final reconstructed 3D image. Data interpolation may be performed for signal superimposition depending on the scanning range and scanning steps.

3. RESULTS AND DISCUSSION

Fig. 2 displays the measurement result of the illumination pattern of the beamsplitting grating at the focal plane of Lens 1 (Fig. 1) measured with a beam profiler (SP620u, Ophir Optronics, USA). Fig. 2(a) displays the intensity distribution and Fig. 2(b) displays the spot size and the distance between two adjacent spots. Note that the spot size and distance are dependent on the effective incident laser beam size and the effective numerical aperture (NA) of the focusing lens employed. In general, with a smaller beam size and/or smaller NA of the lens, the spot size is bigger as can be calculated according to Geometrical Optics theory. In our system, the work distance of Lens 2 is adjustable, meaning that the spot size and FOV can be easily adjusted accordingly. Moreover, since the depth of focus (DOF) is also dependent on the NA of the lens, by adjusting the work distance, the DOF can also be adjusted conveniently. According to our measurement, the DOF is up to ~1 mm with spot size of ~20 μm.

Fig. 3 displays the experimental results of imaging some carbon fibers. Fig. 3(a) displays the reconstructed image from conventional tomography data rendered with maximum intensity projection (MIP) along z axis while Fig. 3(b) displays the reconstructed image acquired with the proposed MSIOAM method. It is clearly seen that the proposed method can improve the spatial resolution dramatically. The ultrasonic array detector has a spatial resolution around 200 μm. As can be seen in Fig. 3(a), with a cluster of small carbon fibers, it is impossible to distinguish them. However, by raster scanning of the sample and signal filtering with virtual pinholes, the reconstructed image can achieve a much higher spatial resolution as shown in Fig. 3(b). Fig. 3(c) displays the 3D volume of the sample in MIP from x-y, y-z and x-z views. It is seen that a large DOF (>1 mm) is attained within the entire FOV. Fig. 3(d) shows the signal plot along the green line indicated in zoomed-in inset of Fig. 3(b). According to the Gaussian fitting results, the system has an effective spatial resolution around 29 μm as characterized by FWHM.

Compared to conventional OAM methods employing a single focused laser spot as the illumination, MSIOAM is a highly parallelized method which can reduce mechanical scanning time and thus more suitable for in vivo studies focusing on fast biodynamics. However, due to the small NA of each mini-beam in MSIOAM, the spot size is usually larger than the aforementioned methods. In order to achieve submicron spatial resolution, additional optical elements such as microscope objective will be required to further reduce the focal spot size. Compared to other parallelized OAM methods such as microlens array based methods, there is no moving part in our system and the long work distance makes it suitable for biological studies. Most importantly, the spatial resolution and FOV can be adjusted easily in MSIOAM. Compared to DMD or spatial light modulator (SLM), beamsplitting grating has a much higher diffraction efficiency and thus more suitable for multifocal illumination strategy.

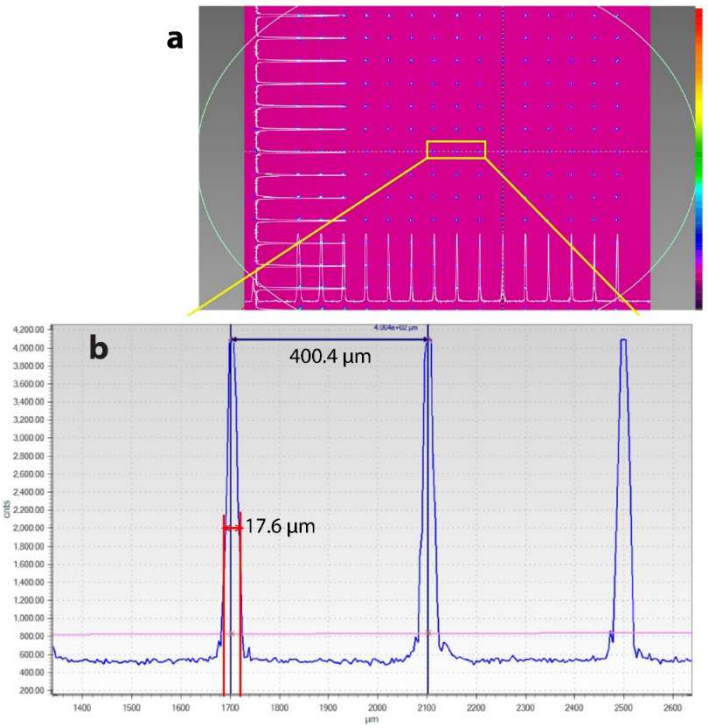

Fig. 2 Illumination intensity distribution measurement of the 15 x 15 beamsplitting grating.

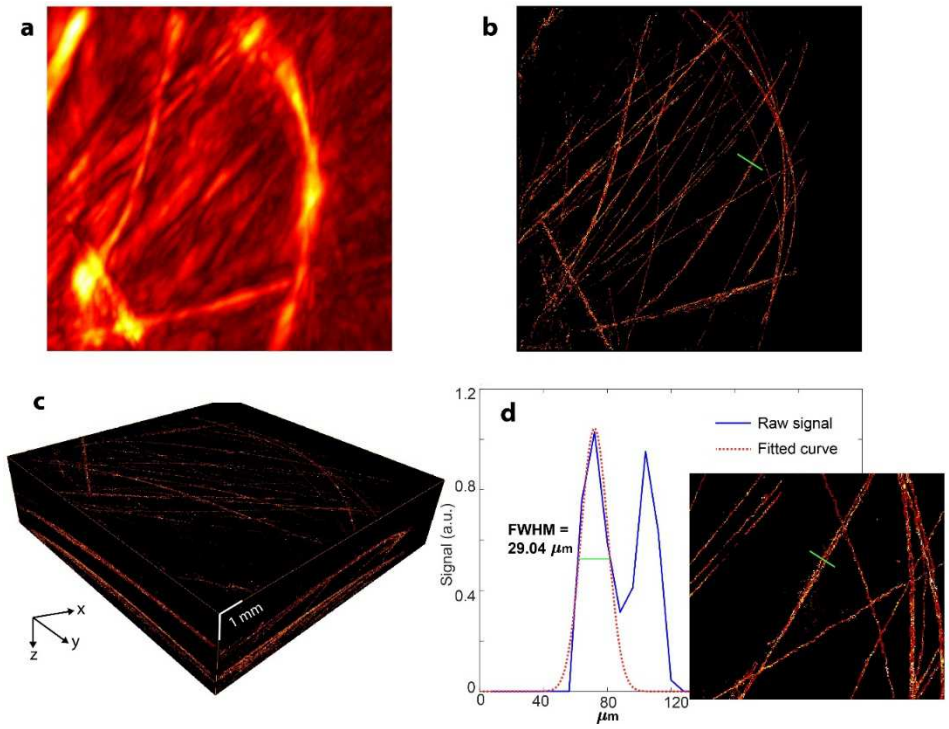

Fig. 3 Experimental results of imaging carbon fibers. (a) MIP image from traditional tomography data. (b) Reconstructed image with the proposed method. (c) Experimental results rendered in 3D. (d) Signal profile along the green line as shown in the zoomed-in inset and (b).

During the phantom experiment, a large volume of data was generated including 10 thousand scanning frames. For each frame, it consisted of 512 channels with 496 samples in each channel. Even state-of-the-art DAQ cannot handle such a large data volume with sampling and quantification at hundreds of frames per second. However, it is practical to greatly increase the frame rate of the ultrasonic array detector up to kHz by reducing the active channels and the sample numbers in each channel [19]. Right now, we are working on faster data acquisition and reconstruction scheme and it is believed that with the proposed method we can achieve real-time high resolution large volume imaging for *in vivo* applications.

4. CONCLUSION

In conclusion, we propose a highly parallelized optoacoustic microscopy method based on multifocal structured illumination and an ultrasonic array detector. By scanning the illumination pattern at hundreds hertz up to kilohertz, fast volumetric optoacoustic imaging in both optical and acoustic resolution modes can be achieved. We believe the proposed method will facilitate many studies looking at functional and dynamic processes, such as neuroimaging, contrast agent uptake, organ perfusion and cell tracking.

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